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Assessment of diastatic, proteolytic and lipolytic activities of yellow and brown varieties of *Cyperus esculentus* (Tigernuts) extracts

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Abstract

Analyses of two varieties of *Cyperus esculentus* (tigernuts) showed that the 100-nut-weight of the yellow variety (49.1 g) was higher than the brown variety (14.8 g). The percentage of moisture contents for the yellow and brown varieties were 13.50% and 5.78% respectively. Treatment of soluble starch with tigernut extracts showed that starch hydrolysis occurred. The time for diastatic activity (α- + β- + γ-amylase activities) to completely hydrolyse starch was generally longer than either α- or β-amylase activity at 50°C. Periods and temperatures for complete starch hydrolysis by α-, β- and γ-amylases were virtually the same in the two tigernut extracts. The shortest time for complete starch hydrolyses by diastatic activity occurred at 50°C and 65°C for both yellow and brown varieties respectively. Least period for starch hydrolysis by α-amylase activity in both varieties occurred at 50°C, while the least time for β-amylase and γ-amylase activities in both tigernut varieties occurred at 65°C. Quantitative determination of amylolytic enzymes of yellow tigernut extract (TNE) on 'dry basis' showed that diastatic activity (183.6º) > α-amylase activity (167.3º) > β-amylase activity (119.8º) > γ-amylase activity (47.5º). Similarly, brown TNE amylolytic enzymes on 'dry basis' showed that diastatic activity (175.8º) > α-amylase activity (140.8º) > β-amylase activity (94.9º) > γ-amylase activity (49.6º). The α-amylase activity in yellow tigernut variety was 1.4-fold that of β-amylase activity but about 1.5-fold in brown variety. However, α-amylase activity (dry basis) was about 3.5-fold that of γ-amylase in yellow variety but 2.8-fold in the brown variety. Extracts from both tigernut varieties also showed proteolytic and lipolytic activities at about 30°C. Evidently, tigernuts contain various endogenous hydrolytic enzymes and the sweetness of tigernut is invariably due to sugars produced from amylase hydrolysis of innate starch.

1. Introduction

Cyperus esculentus commonly called tigernut is available in Nigeria in three different varieties, viz., yellow, brown and black varieties (Adejuyitan, 2011). The yellow variety is usually preferred to the other varieties because of its attractive colour, bigger size, fleshier nuts, more protein content, less anti-nutritional factors like polyphenols and lower fat (Musa and Hamza, 2014; Oladele *et al.,* 2017). Tigernut milk is highly nutritive with high starch, glucose and proteins contents. It is rich in minerals elements (like phosphorous and potassium), and vitamins C and E (Wayah and Shehu, 2015). The fat content of tigernut is relatively similar to those of nuts and seeds such as soya beans, but higher than those of cereals (Sanchez-Zapata *et al.*, 2012). Proximate composition of 100g of raw and processed tigernuts showed a various range of values for moisture content (4.19 – 51.93%), crude protein (2.61 – 10.12%), ash (0.70 – 1.77%), crude fibre (7.48 – 13.97%), carbohydrate (22.73 – 56.85%) and crude fat (10.79 – 32.06%) (Akonor *et al.*, 2019). Tigernut oil contains 18% saturated (palmitic acid and stearic acid) and 82% unsaturated fatty acids (Oleic acid and Linoleic acid) (Zhang *et al.*, 1996).

Plant tubers such as potato (*Solanum tuberosum*), sweet potato (*Ipomoea batatas*) and yam (*Dioscorea esculenta*) are known to contain enzymes, particularly α- and β-amylases which hydrolyse their innate starch content producing sugars that confer sweet taste on them (Beck and Zigler, 1989; Morrison *et al.*, 1993). Given the sweet taste of tigernuts, this work examined two different varieties of *Cyperus esculentus* for the presence

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of endogenous enzymes viz., amylases, proteinases and lipases that hydrolyse intrinsic tigernut starch, proteins and lipids respectively.

2. Materials and methods

2.1 Source of tigernut

Cyperus esculentus varieties: Yellow and brown tigernut varieties were obtained from Jimeta market, Yola, Adamawa State, Nigeria.

2.2 Determination of moisture content

A total of ten randomly chosen tigernuts were weighed. The tigernuts were then dried in an oven at 110°C until constant weight. The percentage of moisture content was determined using the formula

$$\text{Percentage moisture content} = \frac{W_1 - W_2}{W_1} \times 100 \quad (1)$$

Where W1 = Initial weight of 10 nuts, W2 = weight of nuts after drying

2.3 Determination of 100-nut weight

Three different portions, each comprising 10 randomly chosen tigernuts were weighed. The sum of the three weights was divided by 3 and then multiplied by 10 to obtain 100-nut weight.

2.4 Qualitative determination of Amylolytic activity (diastatic activity, alpha activity, beta activity and gamma activity)

2.4.1 Preparation of Tigernut extract (TNE) filtrate

Approximately, 5 g of finely ground tigernut flour using a blender was transferred into 50 mL 0.5% NaCl solution in volumetric flask, mixed thoroughly and then made up to 100 mL mark with 0.5% NaCl solution. This was allowed to stand for 2.5 hrs at room temperature (28 -32°C), with intermittent agitation and rotation every 20 mins. The solution was filtered through 32 cm Whatman fluted filter paper in a 20 mL funnel into a conical flask. The first 10 mL filtrate was returned into the filter and a watch glass was placed over the funnel and the filtrate collected in the flask for 1 hr.

2.4.2 Qualitative determination of diastatic activity

The diastatic activity was determined using a modification of the method described by Owuama (2019). Briefly, 5 mL 2% acetate buffer starch solution was put into each of 8 test tubes in a test tube rack. Then 3 drops of iodine solution were added into each tube and mixed well. 0.5 mL TNE filtrate was then transferred into each of test tubes; 1 to 7, but 0.5 mL H_2O was put into tube-8 (control) and mixed well. All the 8 test tubes in the rack were placed in a water bath at 30°C and monitored for 80 mins or until the blue-black colour disappeared. The process was repeated at different temperatures viz., 50, 55, 60 and 65°C.

2.4.3 Qualitative determination of alpha-amylase activity

Alpha-amylase activity was determined using a modification of the method described by Owuama (2019). Briefly, to 40 mL 2% acetate buffer starch solution in 50 mL volumetric flask, 0.2 mL $HgCl_2$ (1 mg/10 mL) was added (to inactivate β-amylase activity) and mixed thoroughly. Approximately, 5 mL of the mixture was put into each of 8 test tubes (labelled 1 to 8) in a test tube rack. Then 3 drops of iodine solution were added into each tube and mixed well. 0.5 mL TNE filtrate was then transferred into each of tubes; 1 to 7, but 0.5 mL H_2O to tube-8 (control) and mixed well. All the 8 test tubes in the rack were placed in a water bath at 30°C and monitored for 80 mins or until the blue-black colour disappeared. The process was repeated at temperatures 50, 55, 60 and 65°C.

2.4.4 Qualitative determination of beta-amylase activity

Beta amylase activity was determined using a modification of the method described by Owuama (2019). Briefly, to 40 mL 2% buffer starch solution in 50 mL volumetric flask, 0.57 g ammonium oxalate dissolved and thoroughly mixed (to inactivate α-amylase activity). Approximately, 5 mL of the mixture was put into each of 8 test tubes in a test tube rack. Then, 3 drops of iodine solution were added into each tube and mixed well. 0.5 mL TNE filtrate was then transferred into each of tubes 1 to 7 but 0.5 mL H_2O to tube-8 (control) and mixed well. All the 8 test tubes in the rack were placed in a water bath at 30°C and monitored for 80 mins or until the blue-black colour disappeared. The process was repeated at temperatures 50, 55, 60 and 65°C.

2.4.5 Qualitative determination of gamma amylase (amyloglucosidase) activity

Gamma amylase activity was determined using a modification of the method described by Owuama (2019). Briefly, to 40 mL acetate buffer starch solution in 50 mL volumetric flask, 0.57 g ammonium oxalate was dissolved and 0.2 mL $HgCl_2$ (1 mg/10 mL) added and thoroughly mixed (to inactivate α-amylase and β-amylase activities). 5 mL of the mixture was put into each of 8 test tubes in a test tube rack. Then 3 drops of iodine solution were added into each tube and mixed well. 0.5 mL TNE filtrate was then transferred into each of tubes 1 to 7 but 0.5 mL H_2O to tube-8 (control) and mixed well. All the 8 test tubes in the rack were placed

in a water bath at 30℃ and monitored for 80 mins or until the blue-black colour disappeared. The process was repeated at temperatures 50, 55, 60 and 65℃.

2.5 Quantitative determination of amylolytic activity (diastatic activity, alpha activity, beta activity and gamma activity)

2.5.1 Preparation of Tigernut extract (TNE)

Finely ground (with an electric blender), tigernut flour (10 g) was put into 200 mL volumetric flask, then 0.5% NaCl solution was added up to 200 mL and thoroughly mixed. The mixture was allowed to stand for 2.5 hrs at room temperature (28-32℃), with intermittent agitation and rotation every 20 mins. Thereafter, it was filtered through 32 cm Whatman fluted filter paper in a 20 mL funnel into flask. First 10 mL of filtrate was returned to the filter and a watch glass placed over the funnel and collected filtrate in the flask until 1 hr.

2.5.2 Determination of diastatic activity

Diastatic activity of tigernut extract (TNE) was determined using a modified diastatic power method described by Owuama (2019). Briefly, TNE (20 mL) was put into 100 mL volumetric flask, 0.5% NaCl solution was added until it reached the 100 mL mark and then mixed well with the pipette. Then, 10 mL was transferred into 200 mL buffered starch in 250 mL volumetric flask and mixed by rotating flask during the addition. The mixture was allowed to stand at room temperature for 30 mins, then 20 mL 0.5N NaOH was added and rapidly mixed by inverting flask. The volume was made up to 250 mL with distilled water and mixed well. The digested starch solution was labelled – 1 and then used for titration A. The blank correction solution for diastatic activity was prepared as follows. To 10 mL filtered TNE in 250 mL volumetric flask, 20 mL 0.5N NaOH was added and mixed well. Approximately, 200 mL buffered starch solution was then added. The mixture was allowed to stand at room temperature for 30 mins, made up to 250 mL with distilled water, mixed well and then used for titration B.

2.5.3.1 Procedure for titration A

Titration was as described in JECFA (1971), briefly, to 10 mL distilled water in 200 mL conical flask, 10 mL Fehling (or Soxhlet) solution was added and boiled over a gas flame. About ⅔ of the digested starch solution was added from the burette, boiled for 15-20 s while constantly rotating the flask. The liquid was removed from heat when it became blue, more digested starch solution was added and boiled for 10 seconds, and the colour was observed. Boiling was continued for about 2 mins until the blue colour is almost discharged. Then 3 drops 1% methylene blue solution was added (*after* adding methylene blue indicator do not interrupt the boiling). The boiling continued while adding more digested starch solution until one drop completely discharged blue colour (Near the endpoint, the colour is violet lavender. On cooling, the blue colour usually returns). A repeat titration was done by adding at once almost the whole amount of digested starch solution used in previous titration, then the titration was finished off by drop by drop as above. The volume of digested starch (Titre) was record the as 'A'

2.5.3.2 Procedure for titration B (blank correction)

To 10 mL distilled water in 200 mL conical flask, 10 mL Fehling solution was added. The volume of blank correction solution equal to the volume of titre 'A' was added and the mixture boiled. The digested starch solution was then added from the burette, boiled for 15-20 seconds, while constantly rotating the flask. Heat was removed when the liquid was blue, more digested starch solution added and boiled for 10 seconds, and the colour was observed. Boiling was continued for about 2 min until the blue colour is almost discharged. Then 3 drops 1% methylene blue solution was added (after adding methylene blue indicator do not interrupt the boiling). Boiling was continued and more digested starch solution added until one drop completely discharges blue colour (Near the endpoint, the colour is violet lavender. On cooling, the blue colour usually returns). A repeat titration was done by adding at once almost the whole amount of digested starch solution used in the previous titration, then the titration was finished off by drop by drop as above. The volume of digested starch (Titre) was record as 'B'

Calculations for the diastatic power (activity) of TNE in degrees were done by using the formulae below, as stated by JECFA (Food and Agriculture Organization and World Health Organization, 1972).

$$\text{Diastatic Power/activity (As is)} = \frac{5000}{A} \times \frac{B}{A} \quad (2)$$

$$\text{Diastatic Power/activity (Dry basis)} = \frac{(as\ is) \times 100}{100 - M} \quad (3)$$

Where A = mL of digested starch solution used in the direct titration; B = mL of digested starch solution used in the blank titration; M = percent moisture content of tigernut

2.5.4 Determination of alpha-amylase activity

The determination was done using a modified method described by Owuama (2019). TNE filtrate (20 mL) was put into 100 mL volumetric flask, 0.5% NaCl was added until it reached the 100 mL mark and mixed well with a pipette. Approximately, 1 mL (1 mg/10 mL) $HgCl_2$ was added into 200 mL buffered starch solution in

250 mL volumetric flask and mixed by rotating the flask. Then, 10 mL of diluted TNE filtrate was transferred into $HgCl_2$ treated buffered starch and mixed by rotating the flask during the addition. The mixture was allowed to stand at room temperature for 30 mins. 20 mL 0.5 N NaOH was rapidly added and mixed by inverting flask. The volume was made up to 250 mL with distilled water and mix well. The digested starch solution was labelled – 2 and then used for titration A. The blank correction solution for alpha amylase activity was prepared as follows. To 10 mL filtered TNE in a volumetric flask, 20 mL 0.5 N NaOH was added and mixed. 200 mL buffered starch solution containing 1 mL (1 mg/10 mL) $HgCl_2$ was added, mixed well and allowed to stand at room temperature for 30 min. Distilled water was added to make up to 250 mL, mixed well and then used for titration B.

Calculations for the α-amylase activity of TNE in degrees were done by using the formulae below.

$$\alpha - \text{Amylase activity (As is)} = \frac{5000}{A} \times \frac{B}{A} \quad (4)$$

$$\alpha - \text{Amylase activity (Dry basis)} = \frac{(As\ is) \times 100}{100 - M} \quad (5)$$

Where A = mL of digested starch solution used in the direct titration; B = mL of digested starch solution used in the blank titration; M = percent moisture content of tigernut.

2.5.5 Determination of beta-amylase activity

The determination was done using a modified method described by Owuama (2019). TNE filtrate (20 mL) was put into 100 mL volumetric flask, 0.5% NaCl was added until it reached the 100 mL mark and mixed well with a pipette. 2.84 g ammonium oxalate was dissolved in 200 mL buffered starch solution (i.e. 0.1M $(NH_4)_2C_2O_4$) in 250 mL volumetric flask. Then, 10 mL of *diluted* TNE filtrate was transferred into $(NH_4)_2C_2O_4$ treated buffered starch and mixed by rotating the flask during the addition. The mixture was allowed to stand at room temperature for 30 mins. 20 mL 0.5N NaOH was rapidly added and mixed by inverting flask. The volume was made up to 250 mL with distilled water and mix well. The digested starch solution was labelled – 3 and eventually used for titration A. The blank correction solution for beta amylase activity was prepared as follows. To 10 mL filtered TNE in volumetric flask, 20 mL 0.5 N NaOH was added and mixed. 200 mL buffered starch solution containing 2.84 g ammonium oxalate was added, mixed well and allowed to stand at room temperature for 30 mins. Distilled water was added to make up to 250 mL, mixed well and then used for titration B.

Calculations for the β-amylase activity of TNE in degrees were done by using the formulae below.

$$\beta - \text{Amylase activity (As is)} = \frac{5000}{A} \times \frac{B}{A} \quad (6)$$

$$\beta - \text{Amylase activity (Dry basis)} = \frac{(As\ is) \times 100}{100 - M} \quad (7)$$

Where A = mL of digested starch solution used in the direct titration; B = mL of digested starch solution used in the blank titration; M = per cent moisture content of tigernut.

2.5.6 Determination of glucoamylase/ amyloglucosidase/γ-amylase activity

The determination was done using a modified method described by Owuama (2019). TNE filtrate (20 mL) was put into 100 mL volumetric flask, 0.5% NaCl was added until it reached the 100 mL mark and mixed well with a pipette. To 200 mL buffered starch solution in 250 mL volumetric flask, 1 mL (1 mg/10 mL) $HgCl_2$ was added and 2.84 g ammonium oxalate dissolved and rotated to mix well. Then, 10 mL of diluted TNE filtrate was transferred into $(NH_4)_2C_2O_4$-$HgCl_2$ treated buffered starch and mixed by rotating the flask during the addition. The mixture was allowed to stand at room temperature for 30 min. 20 mL 0.5N NaOH was rapidly added and mixed by inverting flask. The volume was made up to 250 mL with distilled water and mix well. The digested starch solution was labelled – 4 and then used for titration A. The blank correction solution for glucoamylase activity was prepared as follows. To 10 mL filtered TNE in a volumetric flask, 20 mL 0.5 N NaOH was added and mixed. 200 mL buffered starch solution containing 1 mL (1 mg/10 mL) $HgCl_2$ + 2.84 g ammonium oxalate was mixed well and allowed to stand at room temperature for 30 min. Distilled water was added to make up to 250 mL, mixed well and then was used for titration B.

Calculations for the γ-amylase activity of TNE in degrees were done by using the formulae below.

$$\gamma - \text{Amylase activity (As is)} = \frac{5000}{A} \times \frac{B}{A} \quad (8)$$

$$\gamma - \text{Amylase activity (Dry basis)} = \frac{(As\ is) \times 100}{100 - M} \quad (9)$$

Where A = mL of digested starch solution used in the direct titration; B = mL of digested starch solution used in the blank titration; M = percent moisture content of tigernut

2.7 Qualitative determination of proteinase activity

Skim milk (0.1 g) was dissolved well in 10 mL 0.5% NaCl. 0.2 mL Folin-Ciocalteaux reagent was added and thoroughly mixed. Then, 5 mL was transferred into each of two clean test tubes, T-1 and T-2 in a test tube rack. 0.5 mL TNE was added to T-1 while 0.5 mL distilled water to T-2 i.e. control experiment. The tubes were

Table 1. Characteristics of Tigernut varieties

Sample	Characteristics					
C. esculentus L.	100-nut wt. (g)	Moisture content (%)	Nut pigment	Iodine into TNE	Proteinase activity	Lipase activity
Yellow variety	49.1	13.5	Yellow	Blue-black	+	+
Brown variety	14.8	5.78	Brown	Blue-black	+	+

Key: + = Present

maintained in a water bath at room temperature (28-32°C) for 16 - 18 hrs and monitored for a change in colour from blue to light yellow in T-1 while no colour change occurred in T-2.

2.8 Qualitative determination of lipase activity

Coconut oil [fat] (0.1 mL) was put into 10 mL 0.1% NaOH and vortexed vigorously for 5 mins to emulsify. Then 3 drops of phenolphthalein indicator were added. 5 mL was transferred into each of two clean test tubes (T-1 and T-2). 0.5 mL TNE was added to T-1 while 0.5 mL distilled water to T-2 i.e. control expt. The tubes were maintained in a water bath at room temperature (28-32°C) for 36 to 48 hrs. Pink colour in tube T-1 disappeared (became colourless apparently due to fatty acids formation), while T-2 remained pink.

3. Results

Yellow and brown varieties of *C. esculentus* were used in this work. The yellow variety had bigger nuts than the brown variety. The average 100-nut weight of yellow variety (49.1 g) was over 3-fold that of brown variety (14.8 g). The percentage moisture content of the yellow variety (13.50%) was higher than that of the brown variety (5.78%). Addition of iodine to tigernut extracts (TNE) gave blue-black colour, indicating the presence of starch (Table 1).

Qualitative analyses of the tigernut extracts from the yellow and brown varieties revealed the presence of diastatic enzymes *viz*., α-amylase, β-amylase and γ-amylase. Periods for complete hydrolysis of starch (as reflected by the loss of blue-black colour), by diastatic enzymes in the two TNE varied with different temperatures (30 to 65°C) (Table 2 and Table 3)

Table 2. Variations in time for starch hydrolysis by diastatic activity, α-amylase, β-amylase and γ-amylase activities at different temperatures by yellow tigernut extract

	Time (min) for loss of blue-black colour of iodine stained starch by yellow TNE			
Temp. (°C)	Diastatic	α-Amylase	β-amylase*	γ-amylase
30	>80	>80	>80	>80
50	35	30	35	75
55	>80	>80	50	60
60	60	65	35	55
65	50	50	30	20

Key: *Blue-black sediments observed at the bottom of the tube after hydrolysis

Table 3. Variations in time for starch hydrolysis by diastatic activity, α-amylase, β-amylase and γ-amylase activities at different temperatures by brown tigernut extract

	Time (min) for loss of blue-black colour of iodine stained starch by brown TNE			
Temp. (°C)	Diastatic	α-Amylase	β-amylase*	γ-amylase
30	>80	>80	>80	>80
50	45	30	30	75
55	>80	>80	50	60
60	>80	75	45	50
65	50	50	25	20

Key: *Blue-black sediments observed at the bottom of the tube after hydrolysis

At 30°C, diastatic activity, α-amylase, β-amylase and γ-amylase activities from both the yellow and brown TNE showed incomplete starch hydrolysis after 80 mins of incubation. However, at 50°C, yellow and brown TNE showed virtually similar hydrolysis pattern viz., complete starch hydrolysis after 35-45 mins of diastatic activity, 30 mins of α-amylase activity, 30-35 mins of β-amylase activity (albeit with blue-black sediments which persisted even after 80 mins) and 75 mins of γ-amylase activity. At 55°C, TNE from both yellow and brown varieties showed similar hydrolysis result i.e. incomplete starch hydrolysis after 80 mins by diastatic activity and α -amylase activity, but complete hydrolysis after 50 mins by β-amylase activity (leaving blue-black sediments) and 60 min by γ-amylase activity. At 60°C, there was remarkable variation in amylolytic activity between the yellow and brown varieties. Except for the γ-amylase activity, the diastatic, α- and β-amylase activities of TNE from yellow variety hydrolysed the starch faster than those from the brown variety. At 65°C, the yellow and brown TNE showed a similar pattern in the period of complete starch hydrolysis for the diastasis (50 mins), α-amylase (50 mins) and γ-amylase (20 mins) activities but a slight difference in the β-amylase activity (25-30 min).

Table 4 shows the variations in the diastatic, α-amylase, β-amylase and γ-amylase activities of TNE from the yellow and brown varieties. In all cases, the 'Dry basis' values were higher than those of 'As is'. Except for the γ-amylase activity, the diastatic activity, a - and β-amylase activities were greater in the yellow TNE than the brown TNE. The brown TNE diastatic activity (165°) [As is], was higher than the α-amylase activity (132.7°), which in turn was greater than the β-amylase activity (89.4º), while γ-amylase had the least activity (46.7°). Similarly, the yellow TNE showed greater diastatic activity (158.8°) [As is], than α-amylase

activity (144.7°), followed by β-amylase activity (103.6º), while γ-amylase activity (41.1°) had the least. The α-amylase activity in yellow TNE was 1.4-fold that of β-amylase activity while it was about 1.5-fold in brown TNE. The α-amylase activity [Dry basis] was about 3.5-fold that of γ-amylase in yellow TNE and 2.8-fold in the brown TNE.

Table 4. Values of various diastatic enzymes from extracts of the two tigernut varieties

Enzymes activity (°)	Yellow variety	Brown variety
Diastatic power (As is)	158.8	165.3
Diastatic power (Dry basis)	183.6	175.4
α-Amylase (As is)	144.7	132.7
α-Amylase (Dry basis)	167.3	140.8
β-Amylase (As is)	103.6	89.4
β-Amylase (Dry basis)	119.8	94.9
γ-Amylase (As is)	41.1	46.7
γ-Amylase (Dry basis)	47.5	49.6

4. Discussion

The higher 100-nut-weight of the *Cyperus esculentus* yellow variety (49.1 g) vis-à-vis the brown variety (14.8 g), and the greater percentage moisture contents of the yellow (13.50%) than the brown (5.78%) variety are consistent with earlier reports (Musa and Hamza, 2014; Akonor *et al.*, 2019). The inherent proteolytic and lipolytic activities in TNE (Table 1), breakdown protein and fat respectively, as well as contribute to starch hydrolysis by removing the protein and lipid on the starch surface thereby enhancing the a- and β-amylases activities. Proteolytic and lipolytic activities have been shown to remove surface proteins and lipids thus quickening enzymatic hydrolysis and *in vitro* digestion of starch (Hu *et al.*, 2018).

The change in colour to blue-black following the addition of iodine to TNE is consistent with the presence of starch in tigernut (Akonor *et al.*, 2019). The loss in blue-black colour from iodine-stained soluble starch treated with TNE of either yellow or brown variety indicates the hydrolysis of starch to sugars by amylases (Izydorczyk and Edney, 2003). TNE of yellow and brown *Cyperus esculentus* varieties have been shown to contain amylases which hydrolyse starch (Avwioroko *et al.*, 2019). The variation in time for starch degradation (i.e. loss of blue-black colour in iodine-stained starch) with temperature by the activities of different amylolytic enzymes, may be attributable to their different modes of action (Zhang *et al.*, 2013), and perhaps the varying effects of inhibitors on the different heat-labile amylases, as well as the antagonism between amylases in digesting cooked starch. Trypsin inhibitors and amylase inhibitors are known to exist in tubers such as sweet potatoes and can be inactivated by heat (Rekha and Padmaja, 2002; Rohn *et al.*, 2002). As well, α-amylase and γ-amylase [α-glucosidase and limit dextrinase] are known to show antagonism during the digestion of cooked starch (Evans *et al.*, 2010; Zhang *et al.*, 2013).

Curiously, the time for starch hydrolysis by diastatic activity (i.e. combined activities of α-, β- and γ-amylases) of both yellow and brown varieties at 50℃, was virtually similar to, instead of being shorter than those of either α- or β-amylase activity. This observation may be attributable to the antagonism between α- and γ-amylases during diastatic activity. Endo-acting α-amylase and exo-acting γ-amylase are known to show antagonistic effects in the digestion of cooked starches but synergistic effects in digesting starch in granular form in the production of glucose (Zhang *et al.*, 2013). Similarly, the antagonism between a-and γ-amylases during diastatic activity, may explain the resemblance in the period for cooked starch hydrolysis between diastatic activity and α-amylase activity at 65℃, but shorter periods for either β- or γ-amylase (amyloglucosidase) activity. Generally, starch hydrolysis by the TNE amylases occurred fastest at 50 and 65℃, about the optimal temperature range for proteinase and amylase activities (Owuama, 1999; Avwioroko *et al.*, 2019). The undigested blue-black sediments observed after β-amylase hydrolysis of boiled soluble starch apparently reflects the inability of the β-amylase to digest the native starch present in TNE containing the crude enzymes (Akonor *et al.*, 2019). It has been shown that native starch granules are not attacked by plant β-amylases without prior digestion by other enzymes or solubilisation (Beck and Zigler, 1989).

The quantitative differences in the activities of various starch digesting enzymes from the TNE varieties revealed that diastatic power/activity > α-amylase > β-amylase > γ-amylase activities and this agrees with the report on sweet sorghum malts (Owuama, 2019). The differences in concentration of various amylase activities may be contributing to variation in hydrolysis potential of β-amylase and α-amylase in tubers (Morrison *et al.*, 1993; Kim *et al.*, 2014). From Table 4, the fact that α-amylase activity is 1.5- to 2-fold higher than the β-amylase activity, and about 2.8- to 3.5-fold greater than γ -amylase activity, compare favourably with reports on tubers (Morrison *et al.*, 1993), and sorghum malts (Owuama, 2019). However, it may be logical to state that the values for α-amylase and β-amylase activities on Table 4 are 'experimental' values, and that the 'actual' values would be deduced from subtracting the γ-amylase activity from each of the α- and β-amylase activities since no substance was added to inhibit γ-amylase activity during the determination of either α-amylase or β -amylase activity. Thus, as the 'experimental' value of α-

amylase activity for yellow variety (dry basis) was 167.3°, the 'actual' value would be 119.8° (i.e. 167.3° minus 47.5°).

In conclusion, tigernut extracts contain endogenous enzymes including proteinases, lipases and different amylases which hydrolyse the innate components of tigernut. Except for the γ-amylase activity, the diastatic activity, α-amylase and β-amylase activities were higher in the yellow than in the brown TNE. Apparently, the innate amylases in tigernut, particularly β-amylase, hydrolyse its native starch to produce maltose which contributes to the sweetness of tigernut, as has been reported for sweet potato and ripe fruits (Morrison *et al.*, 1993). The activities of TNE proteolytic and lypolytic enzymes on starch surface proteins and lipids apparently influence starch hydrolysis by amylases. Given the range of enzymes contained in TNE, it is conceivable that certain foods which are products of enzyme modification can be processed by amending their precursor with tigernuts flour or TNE.

Conflict of interest

There is no conflict of interest.

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